15

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Applicants	:	Qiu et al.	Examiner: A. Kubelik
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For	:	ENHANCEMENT OF GROWTH IN PLANTS	

DECLARATION OF ZHONG-MIN WEI UNDER 37 C.F.R. § 1.132

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

I, ZHONG-MIN WEI, pursuant of 37 C.F.R. § 1.132, declare:

1. I received a B.S. degree in Biology from Zhejiang University, Zhejiang, China in 1982, an M.S. degree in Plant Pathology from Nanjing Agricultural University, Nanjing, China in 1984, and a Ph.D. degree in Molecular Biology from Nanjing Agricultural University and Academy of Science, Shanghai, China in 1987.

2. I am currently employed as Chief Scientific Officer and Vice President of Research and Development at EDEN Bioscience Corporation in Bothell, Washington.

3. I am an inventor of the above-identified application.

4. I am presenting this declaration to show that plants transformed with a DNA molecule encoding a hypersensitive response elicitor exhibit enhanced growth when compared to plants not transformed with a hypersensitive response elicitor. In addition, I am presenting this declaration to show that topical treatment of plants with hypersensitive response elicitors from a variety of sources is effective to enhance growth of the plants.

Enhancement of Growth With Transgenic Plants Encoding a Hypersensitive Response Elicitor

5. As demonstrated by the following experimental evidence in paragraphs 6-8 below, both Arabidopsis and cotton plants grown from seeds harvested from plants that had been transformed with a DNA molecule encoding the HrpN hypersensitive response elicitor from *Erwinia amylovora* exhibited enhanced growth.

6. In order to determine if transforming plants or plant seeds with a DNA molecule encoding a hypersensitive response elicitor resulted in enhanced plant growth, transformation constructs containing the *hrpN* gene from *Erwinia amylovora* were generated. The basic *hrpN* transformation constructs included the *hrpN* gene inserted behind a NOS promoter (NOSP), followed by a NOS terminator (NOST). The NOS promoter is from the nopaline synthase gene and is considered a weak constitutive promoter.

The NOS Promoter Sequence is as follows:

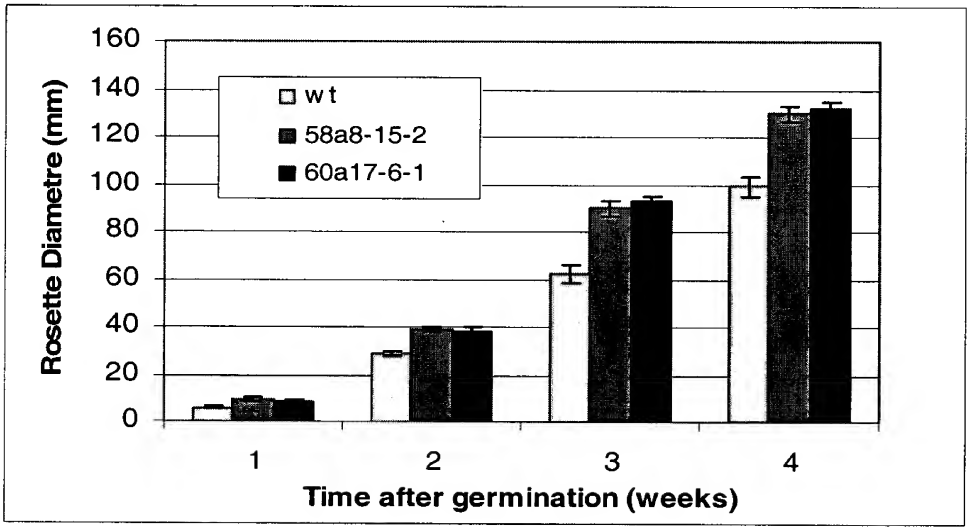
AAGCTTCCCAAACTGAAGGCGGGAAACGACAATCTGATCATGAGCGGAGAATTA
AGGGAGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGTTTTACGTTTG
GAACTGACAGAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGAGT
TTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCT
AAGGTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGACGTTCC
ATAAATTCCCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAACCAAATAAT
CTGCACCGGATCC

A second construct was assembled that inserted the tobacco *pr1b* signal sequence (SS) between the NOS promoter and *hrpN* gene. The nucleotide sequence of the signal sequence is as follows:

ATGGGATTTTTTCTCTTTTCACAAATGCCCTCATTTTTTCTTGTCTCTACACTTCTC
TTATTCCTAATAATATCTCACTCTTCTCATGCC

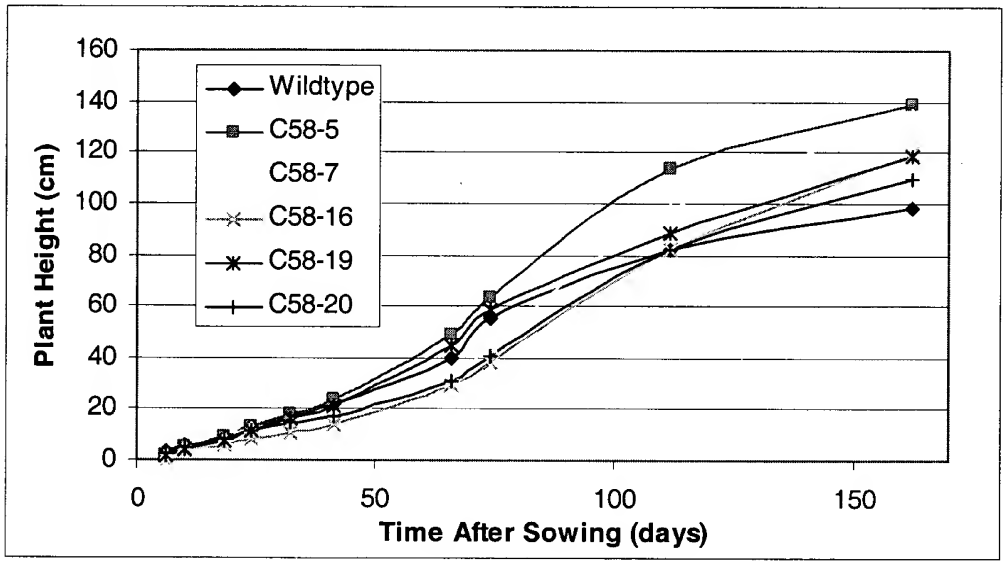
7. Arabidopsis Col-0 plants were transformed with the transformation constructs described above. The constructs were transformed by standard procedures utilizing *Agrobacterium*-mediated transformation. Plants designated 58a8-15-2 were transformed with the construct that contained the basic *hrpN* transformation construct with no signal sequence. Plants designated 60a17-6-1 were transformed with the construct that included the *pr1b* signal sequence. High *hrpN* expression transgenic lines were selected by Northern analysis. The lines were confirmed to be homozygous by selection on Kanamycin. Prior to initiation of the growth assays, the seeds of each transgenic line and the wild type (wt) Arabidopsis were sterilized and subjected to a vernalization treatment in which the seeds were placed at 4°C for approximately four days. The vernalization treatment aided in synchronizing the germination of the wild type and transgenic Arabidopsis seeds. All seeds and plants were maintained in identical conditions: 16 hours daylight period, 23 C (day)/ 20 C (night), and 50% humidity. Plant growth was evaluated by measuring the diameter of the leaf rosette at different times during the plant's development. Twelve plants were evaluated for each transgenic line and the wild type Arabidopsis control group. The average rosette diameter and the standard error within each group were calculated and are shown below in the Figure 1. Both *hrpN* transgenic Arabidopsis lines showed increased growth of approximately 23% over non-transgenic Arabidopsis.

Figure 1. Rosette Diameter of *hrpN* Transgenic Arabidopsis and Wild Type Arabidopsis



8. Cotton plants (Coker 312-5a) were transformed with the basic *hrpN* transformation construct that did not contain a signal sequence. The cotton plants were transformed by standard procedures utilizing *Agrobacterium* transfection and NPT2 selection. The plants evaluated were grown from the seed collected from independently regenerated transgenic plants and were designated; C58-5, C58-7, C58-16, C58-19, and C58-20. Plants were maintained in a 14 hours daylight period at 25 C (day)/ 22 C (night). Plant growth was evaluated by measuring the plant height at different times during the plant's development. Approximately ten plants were evaluated for each transgenic and wild type group. The average heights of each group are shown below in the Figure 2. *hrpN* transgenic cotton showed increased growth of approximately 23% over non-transgenic cotton plants

Figure 2. Plant Height of *hrpN* Transgenic Cotton and Wild Type Cotton



Enhancement of Growth By Topical Treatment of Plants with *Pseudomonas syringae* and *Xanthomonas campestris* Hypersensitive Response Elicitors

9. As demonstrated by the following experimental evidence in paragraphs 10-11 below, topical application of hypersensitive response elicitors from a range of sources, such as *Psuedomonas syringae* (HrpZ) and *Xanthomonas campestris* (HreX), enhances the growth of plants.

10. The hypersensitive response elicitor HreX from *Xanthomonas campestris* was evaluated for induction of plant growth enhancement. Prior to sowing,

tomato seeds were soaked for approximately four hours in either a solution containing the partially purified HreX protein diluted in potassium-phosphate buffer, or potassium-phosphate buffer alone. The pre-treated seeds were then planted and maintained in identical conditions in a controlled environment. Each treatment group consisted of 3 pots, each pot containing 8 plants. The average plant heights and percent differences between the treatment groups are shown below in Table 1. As these results demonstrate, plants treated with HreX grew substantially more than the buffer-treated control plants.

Table 1. Growth Enhancement from Treatment of Tomato with the Hypersensitive Response Elicitor HreX.

Treatment Groups	Replicates[1]			Mean[2]	% Difference
	Pot #1	Pot #2	Pot #3		
HreX	7.4	7.3	6.8	7.1a	15.5
Buffer Control	6.1	6.1	5.6	6.0b	na

[1] Mean height of the 8 plants in each pot.
[2] Means followed by the same letter do not significantly differ (P=0.01, LSD)

11. The hypersensitive response elicitor HrpZ from *Psuedomonas syringae* was evaluated for induction of plant growth enhancement. Prior to sowing, tomato seeds were soaked for approximately four hours in either a solution containing the partially purified HrpZ protein diluted in potassium-phosphate buffer or potassium-phosphate buffer alone. The pre-treated seeds were then planted and maintained in identical conditions in a controlled environment. Each treatment group consisted of 6 pots, each pot containing 10 plants. The average plant heights and percent differences between the treatment groups are shown below in Table 2. As these results demonstrate, plants treated with HrpZ grew substantially more than the buffer-treated control plants.

Table 2. Growth Enhancement from Treatment of Tomato with the Hypersensitive Response Elicitor HrpZ.

Treatment Groups	Replicates[1]						Mean[2]	% Difference
	Pot #1	Pot #2	Pot #3	Pot #4	Pot #5	Pot #6		
HrpZ	5.10	5.28	4.60	4.72	4.71	4.87	4.88a	9.6
Buffer Control	4.15	4.38	3.84	4.31	4.62	5.18	4.41b	na

[1] Mean height of the 18 to 21 plants in each pot.
[2] Means followed by the same letter do not significantly differ (P=0.054, LSD)

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that theses statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 9/25/02

Zhong-Min Wei